COMPARATIVE STRUCTURAL STABILITY OF HISTONES BY INTERACTION OF SODIUM N-DODECYL SULPHATE

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Abstract

The interaction of sodium n-dodecyl sulphate (SDS) with histones in phosphate buffer pH 6.4 has been studied spectroscopically and by equilibrium dialysis. The enthalpies of interaction and the spectroscopic data suggest different structural stability for histones-SDS interaction. The linear relation between enthaly and absorbtion was determined by the equation of $m=\frac{\Delta H-\Delta H_{max}}{A_{max}-A}$, where m is the stability coefficient of histones-SDS interaction indicating the relationship of the structural stability of histones-SDS complexes.

Introduction

The nuclei of the cells of higher organisms are known to contain approximately equal amounts of DNA, basic proteins, histones and a smaller quantity of non-histone proteins. The function of DNA is well understood, while the role of the protein components remains obscure.

Two major functions have been ascribed to histones; one is that histones play a structural role causing the coiling of DNA to compact the chromosome; the second function is that histones are involved in the regulation of genetic activities. The histones are also believed to be responsible for the suppression of genetic activities in differentiated tissues [1,2].

Earlier physical studies of histones were concerned with a mixture of histones. Today, we know that histones are not only heterogeneous, but they are also being classified into five major fractions called H1, H2A, H2B, H3 and H4 [3].

In view of the increased interest in the functions of histones, we have concerned ourselves with denaturation studies of the interaction between sodium n-dodecyl sulphate (SDS) and histones [4-6].

The object of this work was to study the conformational stability of histones by using SDS as a denaturant, and to determine the thermodynamic and spectroscopic changes accordingly.

Keywords: Stability of histones; Sodium n-dodecyl sulphate; Enthalpy; Spectroscopy

Experimental Section

Materials

Histones were obtained from Sigma Chemical Co. Ltd. Sodium n-dodecyl sulphate (especially pure grade) was used as it was supplied by Merck. All other materials and reagents were of analytical grades. The solutions were made up with double-distilled water. The composition of the buffer used was 2.5 mM sodium phosphate (pH 6.4, I = 0.0069) containing 0.02% (w/v) sodium azide contributing 0.0031 to the ionic strength

(I). Rosaniline hydrochloride dye was used as it was supplied by B.D.H. Visking membrane dialysis tubing (molecular weight cut off 10000-14000) was obtained from SIC (East Leigh) Hampshire, U.K.

Methods

The enthalpy and the absorbtion measurements were made with equilibrium dialysis and Shimadzu U.V. spectrophtometer, model 160 respectively [6]. The concentration of histones was 0.01% w/v. In all calculations the molecular weight of H1, H2A, H2B and H3 were taken as 21,000, 14,000, 13750 and 15300, respectively [7].

Results and Discussion

Figure 1 shows the enthalpies of interaction of histones (H1, H2B, H3A and H3) with SDS as obtained from van't Hoff equation according to our previous reports [4,5,6, and 8]. The upper axis of the enthalpy curve shows the number of SDS ions bound per molecule of protein ($\bar{\nu}$) as measured by equilibrium dialysis. The lower axis shows the final concentration of SDS in solution. The curves illustrate the differences in enthalpies of interaction for various SDS-histones complexes.

For ($\bar{\nu}$) = 30 the amounts of endothermic enthalpy (ΔH) is equal to 2,000, 1,000, 600 and 300, and $\Delta H_{\bar{\nu}}$ is equal to ~ 66, 33, 20 and 10 K_J mol^{-1} for H3, H2A, H2B and H1 histones respectively. The magnitude of ΔH and $\Delta H_{\bar{\nu}}$ show the extent of denaturations of H3, H2A, H2B and H1 histones. The SDS concentration at which the binding to histones saturates are approximately 0.5, 0.8, 1.5 and 1.7 mM (assuming saturation binding correspond to ~ 1.4 g SDS g^{-1} of Protein) [9-11] for H3, H2A, H2B and H1 histones respectively. At these SDS concentrations, the enthalpies of interaction are 4,000, 3,000, 2,800, and 1,000 K_J mol^{-1} for H3, H2A, H2B and H1 histones respectively. The corresponding $\bar{\nu}$ for saturation of histones are 80, 75, 70 and 100; and $\Delta H_{\bar{\nu}}$ are 50, 42.2, 40 and 10 K_J mol^{-1} for H3, H2A, H2B and H1 histones respectively. $\Delta H_{\bar{\nu}}$ is an indicator of the binding affinity of histone - SDS complexes.

The SDS binding affinities for H_3 and H_1 histones are quite different, while the binding affinities of H2A and H2B histones are quite similar. Since SDS concentrations employed were below the critical micelle concentration (cmc); therefore the thermal correction was not required.

Figure 2 is a plot of the absorbtion of the histone solutions as a function of the final concentration of SDS. The plots are obtained by adding portions of SDS solutions to the histone solutions at $OD_{225\,nm}$. The plots show an initial steep rise reaching the maxima and then causing hypochromism. A_{max} are at $\bar{\nu}$ equal to ~ 40, 20, 30, 40 for H3, H2A, H2B and H1 respectively. The effect of SDS on the change in absorbance of

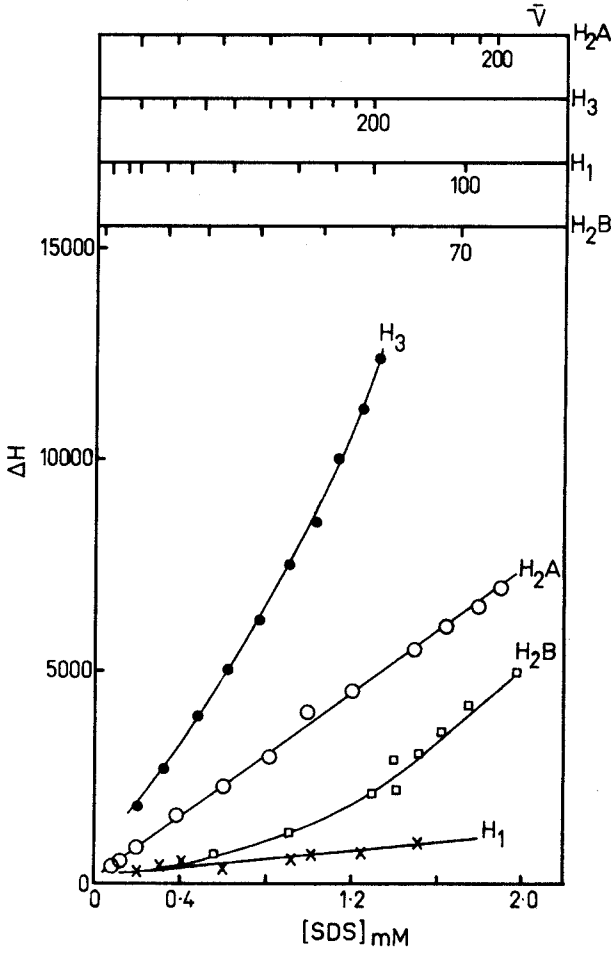

Figure 1. Enthalpy of interaction between histones and SDS in phosphate buffer, pH 6.4 betwen 27° and 37°C. The upper axis shows the number of SDS molecules bound per histones molecule at equilibrium.
●, H3; ○, H2A ❑, H2B; ×, H1

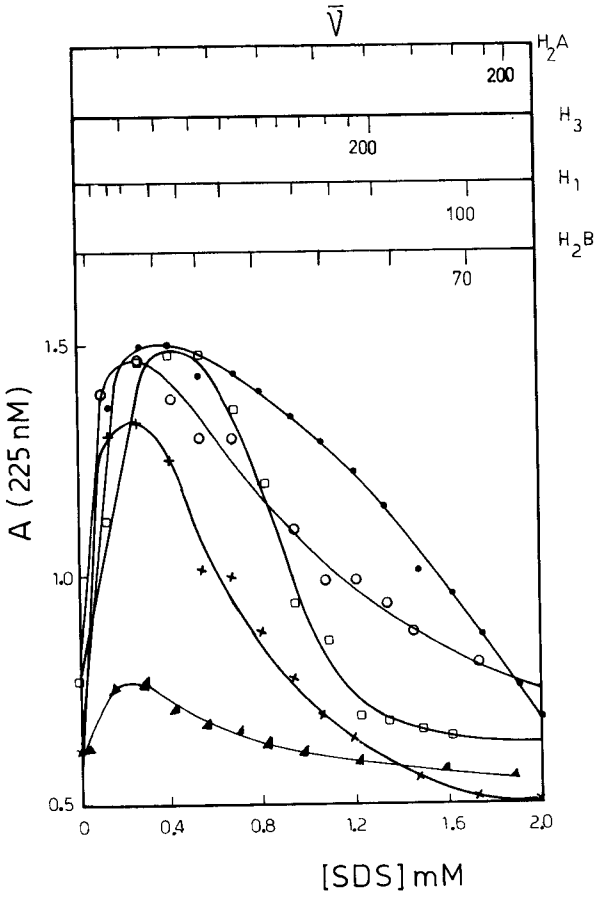

Figure 2. Absorbtion changes of the interaction between histones and SDS in phosphate buffer, pH 6.4 at 27°C. The upper axis shows the number of SDS molecules bound per histone molecule at equilibrium.
●,H3; ○,H2A; ❑,H2B; ×,H1: ▲,WH

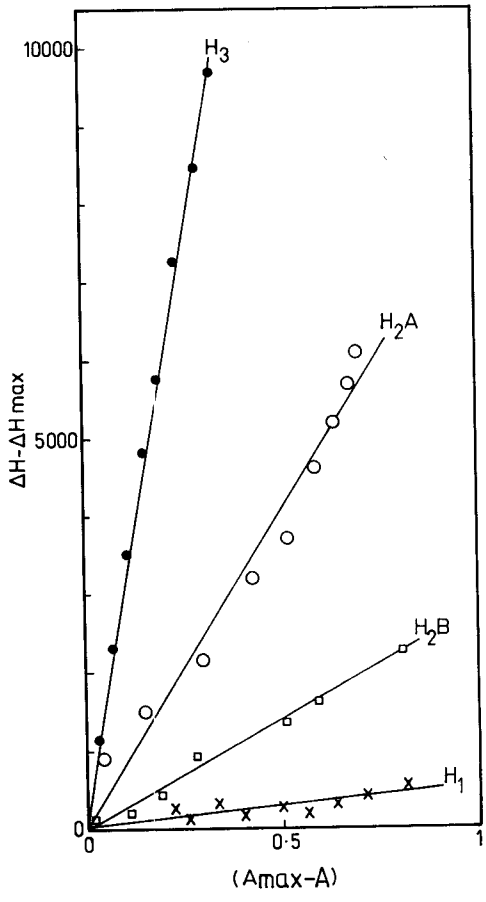

Figure 3. The linear relationship of ($\Delta H-\Delta H_{max}$) versus ($A_{max}-A$)
●,H3; ○,H2A; ❑,H2B; ×▲,H1

histones was different. The distinct variation occurred for H3-SDS complexes. The plots show fairly similar variation for interaction of SDS with H2B and H2A. This close similarity was confirmed by thermodynamic parmeter of ΔH_{v} which was discussed previously. It is important to note that the amino acid sequence of H2B and H2A histones are quite similar [12]. The absorbtion curve for H_1- SDS complexes was stated under the H3, H2A and H2B histones which corresponds to the enthalpy curve in Fig.1. Finally, Figure 2 shows the small variation for the interaction of SDS with whole histones (WH) which is indicative of more stability of WH, compared to histone fractions; according to the reasons already mentioned, the association of histones fractions might be the cause of the stability of the structure of these histones.

To obtain the relation of enthalpy and absorbtion data for histone-SDS complexes, ($\Delta H-\Delta H_{max}$) is plotted as a function of (A_{max} -A) which is shown in Figure 3. The equation of $m = \frac{\Delta H-\Delta H\,max}{A-A_{max}}$ is constructed to determine the linear relationship between enthalpy and absorbtion for the interaction of SDS and histones, where m is the slope of the linear plots in Figure 3 designating the stability of histone-SDS interaction. The quantity of m is equal to 27,500; 8,000; 3,000 and 500 for H3, H2A, H2B and H1 respectively.

In conclusion, the calculated data strongly support the structural stability for the histone- SDS interaction follow as WH>H1 > H2B > H2A >H3. Based on our findings we suggest that the SDS interaction technique is a successful method for comparison of the structure of

proteins.

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References

1. I. Isenberg. *Ann. Rev. Biochem.* 48,159 (1979).
2. A. Csordas, *Biochem. J.* 265,23 (1990).
3. S.B., Roberts, W. Emmons and G., Childs, J. *Mol. Biol.* 196, 27(1987).
4. A.A. Moosavi-Movahedi, A. Rabbani, M. Goodarzi and B. Goliaei, *Thermochimica Acta* 154, 205 (1989).
5. A.A. Moosavi-Movahedi and M.R. Housaindokht, *Int. J. Biol Macromol.* 13, 50 (1991).
6. A.A. Moosavi-Movahedi, M. Goodarzi and M.R. Housandokht, *J.Sci, I.R. Iran.* 1,81 (1990).
7. K., Hamana and K., Iwai, J. *Biochem.* 76, 503 (1974).
8. A.A. Moosavi-Movahedi, M.R. Housaindokht, Physiological Chem. Physics and Medical Nmr. 22,19 (1990).
9. A.A. Moosavi-Movahedi, M.N. Jones and G. Pilcher, *Int. J. Biol. Macromol.* 10,75 (1988).
10. A.A. Moosavi-Movahedi, M.N. Jones and G. Pilcher, *ibid* 11,26 (1989).
11. R. Pitt-Riverse and F.S.A., Impiombato, *Biochem.* J. 109, 825 (1968).
12. L.S. Hnilica, The Structure and Biological Functions of Histones. CRC press, Cleveland, Ohio 1972.